

CLAIMS

1. Method of obtaining dendritic cells, characterized in that it consists in:
- 5 1) cultivating, for 4 to 6 days, mononuclear cells derived from cytopheresis after mobilization, in a serum-free medium supplemented with human albumin, in the presence of a granulocyte-macrophage colony stimulating factor (GM-CSF) and an interleukin (IL) that blocks differentiation towards the macrophagic pathway;
- 10 2) adding TNF- α and optionally an inflammatory mediator to the culture medium and continuing the culture for about a further 1 to 4 days; and
- 3) recovering the dendritic cells formed.
2. Method according to claim 1, characterized in that the culture of step 1) is carried out for 5 days and that of step 2) for 2 days.
3. Method according to claim 1 or 2, characterized in that the interleukin is
- 15 interleukin-4 or interleukin-13.
4. Method according to any one of claim 1 to 3, characterized in that the inflammatory mediator is tumor necrosis factor alpha (TNF- α).
5. Method according to any one of claims 1 to 3, characterized in that the inflammatory mediator is tumor necrosis factor alpha (TNF- α) and
- 20 prostaglandin E2 (PGE2).
6. Method according to any one of claims 1 to 5, characterized in that the mononuclear cells are obtained by cytopheresis after mobilization by chemotherapy and/or with at least one cell growth factor.
7. Method according to any one of claims 1 to 6, characterized in that GM-CSF, interleukin and TNF- α are each used at a rate of 1 to 1000 ng/ml of
- 25 medium.
8. Method according to any one of claims 1 to 7, characterized in that human albumin is used at a rate of 1 to 2% (weight/volume of medium).
9. Method according to any one of claims 1 to 8, characterized in that human
- 30 albumin is used at a rate of 2% (weight/volume of medium).
10. Irreversible dendritic cells, characterized in that they are $\alpha v\beta 3^-$, $\alpha v\beta 5^+$, CCR5 $^-$ and CCR7 $^+$.
11. Use of $\alpha v\beta 3^-$, $\alpha v\beta 5^+$, CCR5 $^-$ and CCR7 $^+$ irreversible dendritic cells for the
- 35 preparation of an immunotherapeutic agent useful for the treatment of any disease involving the immune system.

12. Method of immunotherapeutic treatment, characterized in that it consists in:
- 1) taking mononuclear cells from a patient to be treated by cytopheresis after mobilization by chemotherapy and/or with a cell growth factor and optionally freezing/thawing;
 - 5 2) cultivating, for 4 to 6 days, mononuclear cells derived from cytopheresis after mobilization, in a serum-free medium supplemented with human albumin, in the presence of a granulocyte-macrophage colony stimulating factor (GM-CSF) and an interleukin (IL) that blocks differentiation towards the macrophagic pathway;
 - 10 3) adding $\text{TNF-}\alpha$ and optionally an inflammatory mediator to the culture medium and continuing the culture for about a further 1 to 4 days while activating them with specific antigens;
 - 4) recovering the dendritic cells formed and activated in this way; and
 - 5) reinjecting said dendritic cells into said patient.
- 15 13. Method according to claim 12 characterized in that said dendritic cells are frozen/thawed before being reinjected into said patient.

AdAⁿ